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SWI/SNF Binding to the *HO* Promoter Requires Histone Acetylation and Stimulates TATA-Binding Protein Recruitment

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We use chromatin immunoprecipitation assays to show that the Gcn5 histone acetyltransferase in SAGA is required for SWI/SNF association with the *HO* promoter and that binding of SWI/SNF and SAGA are interdependent. Previous results showed that SWI/SNF binding to *HO* was Gcn5 independent, but that work used a strain with a mutation in the Ash1 daughter-specific repressor of *HO* expression. Here, we show that Ash1 functions as a repressor that inhibits SWI/SNF binding and that Gcn5 is required to overcome Ash1 repression in mother cells to allow *HO* transcription. Thus, Gcn5 facilitates SWI/SNF binding by antagonizing Ash1. Similarly, a mutation in *SIN3*, like an *ash1* mutation, allows both *HO* expression and SWI/SNF binding in the absence of Gcn5. Although Ash1 has recently been identified in a Sin3-Rpd3 complex, our genetic analysis shows that Ash1 and Sin3 have distinct functions in regulating *HO*. Analysis of mutant strains shows that SWI/SNF binding and *HO* expression are correlated and regulated by histone acetylation. The defect in *HO* expression caused by a mutant SWI/SNF with a Swi2(E834K) substitution can be partially suppressed by *ash1* or *spt3* mutation or by a gain-of-function V71E substitution in the TATA-binding protein (TBP). Spt3 inhibits TBP binding at *HO*, and genetic analysis suggests that Spt3 and TBP(V71E) act in the same pathway, distinct from that of Ash1. We have detected SWI/SNF binding at the *HO* TATA region, and our results suggest that SWI/SNF, either directly or indirectly, facilitates TBP binding at *HO*.

The *Saccharomyces cerevisiae HO* gene encodes an endonuclease that initiates mating-type switching in haploid yeast cells, and the gene is governed by complex transcriptional regulation (for reviews, see references 22, 41, and 62). The gene is expressed only during the late G_1 phase of the cell cycle, and only in mother cells, one of the two progeny after mitotic division. The Ash1 repressor protein is required for this asymmetric expression, as *HO* is expressed in both mother and daughter cells in an *ash1* mutant (14, 76).

Chromatin structure plays an important role in transcriptional regulation, including at *HO*. There are two major classes of chromatin-modifying factors that alleviate the repressive effects of chromatin, the ATP-dependent chromatin-remodeling factors, such as SWI/SNF, and histone acetyltransferases (HATs) that covalently modify the N-terminal tails of histones by acetylation (84). Recent work has shown that transcription factors recruit chromatin-modifying factors to promoters and that at some promoters, the concerted action of chromatin-remodeling and HAT complexes is required for gene activation (61). It has been shown for a number of promoters that sequence-specific DNA-binding proteins recruit chromatin remodelers and HATs in a temporal order.

Sequential recruitment of transcription factors was first shown at the *HO* gene (25). *HO* contains two defined upstream promoter regions, URS1 and URS2, which contain recognition sites for the Swi5 and SBF sequence-specific DNA-binding factors, respectively, as well as a TATA region (Fig. 1A). Chromatin immunoprecipitation (ChIP) experiments have shown that the first event in *HO* activation occurs in late anaphase, when the Swi5 activator enters the nucleus and binds to the far-upstream URS1 region of the *HO* promoter (25). Swi5 interacts directly with SWI/SNF (66), and Swi5 is required for the subsequent recruitment of SWI/SNF. The Mediator complex then binds to the URS1 region, long before the arrival of RNA polymerase II (Pol II) (12). There is a direct interaction between Mediator and Swi5, and genetic experiments have shown that both Swi5 and SWI/SNF are required for Mediator recruitment (12). The SAGA complex containing the Gcn5 HAT binds next, and a mutation in the *SWI2* subunit of the SWI/SNF complex prevents SAGA binding (25). Acetylation of histones H3 and H4 at the *HO* promoter has been observed in early G_1 phase of the cell cycle, and this acetylation requires the Gcn5 histone acetyltransferase (53). Next, the SBF factor, composed of two subunits encoded by the *SWI4* and *SWI6* genes, binds to its sites in URS2 (25). Thereafter, Mediator binds to the TATA region, dependent on the SBF factor. Activation of the Cdc28 cyclin-dependent kinase is required for binding of RNA polymerase, TFIIB, and TFIIH at the *HO* TATA (24).

Ordered recruitment of transcription factors has also been seen at other promoters (for a review, see reference 23), including the human beta interferon, α1 antitrypsin, collagenase, and PPARγ2 promoters (2, 58, 73, 77). For example, at the beta interferon promoter, binding of sequence-specific factors results in the sequential recruitment of the Gcn5 complex, followed by the CBP-RNA Pol II complex and then the SWI/SNF complex (2). The order of factor recruitment observed here differs from that seen at *HO*. At the yeast *HO* gene, the SWI/SNF chromatin remodeler binds at an early step, while the SWI/SNF remodeler acts very late

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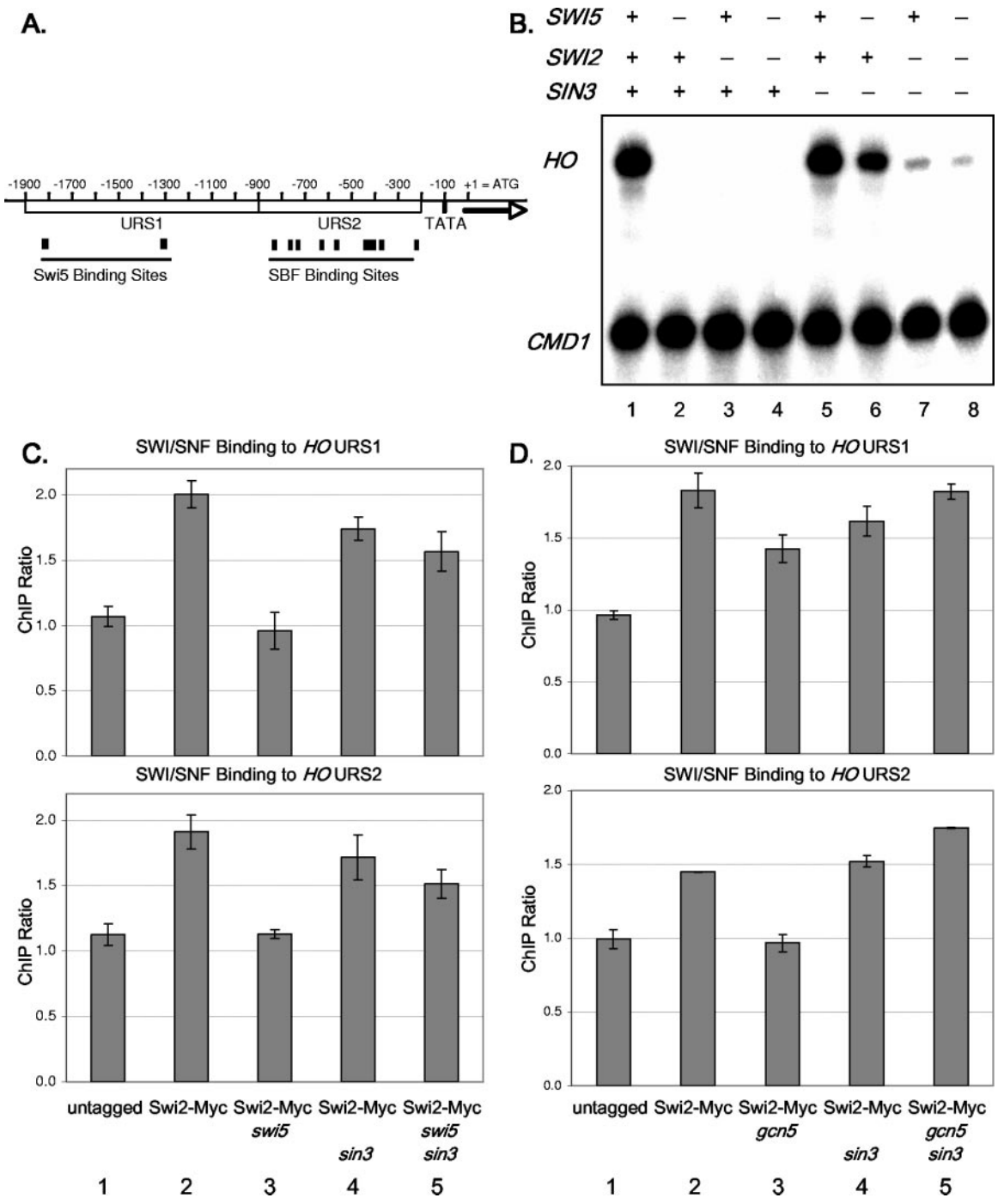


FIG. 1. SWI/SNF binding to *HO* is restored in the *swi5 sin3* mutant. (A) Map of the *HO* promoter showing positions of URS1, URS2, and TATA. (B) *HO* is expressed in the *swi5 sin3* mutant. RNAs were prepared from strains DY150, DY408, DY5270, DY3498, DY984, DY986, DY2870, and DY3499 and used for S1 nuclease protection assays to measure *HO* and *CMD1* (internal control) RNA levels. (C) ChIP was performed with an untagged strain (DY150) and with Swi2-Myc strains that were wild type (DY6151), *swi5* (DY9395), or *swi5 sin3* (DY9391). SWI/SNF binding to either *HO* URS1 or URS2 was measured by real-time PCR, and the units are arbitrary after normalization to a *YDL224c* internal control. The error bars show the standard deviations of the ChIP PCRs performed in triplicate. (D) SWI/SNF binding to *HO* is restored in a *gcn5 sin3* strain. ChIP was performed with an untagged strain (DY150) and with Swi2-Myc strains that were wild type (DY6151), *gcn5* (DY8738), *sin3* (DY9923), or *gcn5 sin3* (DY9927). SWI/SNF binding to either *HO* URS1 or URS2 was measured by real-time PCR, and the units are arbitrary after normalization to a *YDL224c* internal control. The error bars show the standard deviations of the ChIP PCRs performed in triplicate.

at the human beta interferon promoter, apparently inducing gene transcription by remodeling the nucleosome near the TATA element and transcriptional start site (56). Similar results are seen at the α1 antitrypsin, collagenase, and PPARγ2 promoters, where binding of HATs and histone acetylation precede binding of SWI/SNF and SWI/SNF functions late in the activation of these genes, often with SWI/SNF binding after polymerase, Mediator, or basal factors have associated with the promoter. It has been shown that

TABLE 1. Strain list

Strain	Description
DY150	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*
DY408	*MAT***a** *swi5*::*hisG-URA3-hisG ade2 can1 his3 leu2 trp1 ura3*
DY984	*MAT***a** *sin3*::*ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY986	*MAT***a** *swi5*::*hisG-URA3-hisG sin3*::*ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY2870	*MAT***a** *sin3*::*LEU2 swi2*::*ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY3498	*MAT*α *swi2*::*ADE2 swi5*::*hisG-URA3-hisG ade2 can1 his3 leu2 trp1 ura3*
DY3499	*MAT***a** *swi2*::*ADE2 sin3*::*LEU2 swi5*::*hisG-URA3-hisG ade2 can1 his3 leu2 trp1 ura3*
DY4394	*MAT***a** *ash1*::*LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY5265	*MAT***a** *gcn5*::*TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5268	*MAT***a** *gcn5*::*TRP1 ash1*::*LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5269	*MAT*α *gcn5*::*TRP1 ash1*::*LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5270	*MAT***a** *swi2*::*ADE2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY5297	*MAT***a** *gcn5*::*TRP1 sin3*::*ADE2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY6151	*MAT***a** *SWI2-Myc*::*TRP1 ade2 can1 his3 leu2 trp1 ura3*
DY6394	*MAT***a** *sin3*::*ADE2 ash1*::*LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY6806	*MAT***a** *spt3*::*ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY7131	*MAT***a** *ash1*::*LEU2 spt3*::*ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY7158	*MAT*α *hmr*Δ::*URA3 ade2 can1 his3 leu2 trp1 ura3*
DY7242	*MAT***a** *spt15*::*LEU2* + *SPT15*(YCp-*URA3-ADE3*) *ade2 ade3 can1 his3 leu2 trp1 ura3*
DY7385	*MAT***a** *gcn5*::*HIS3 sin3*::*ADE2 ash1*::*LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY7387	*MAT***a** *gcn5*::*HIS3 ash1*::*LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY7403	*MAT***a** *SWI2-Myc*::*TRP1 ash1*::*LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY8736	*MAT***a** *SWI2-Myc*::*TRP1 ash1*::*LEU2 gcn5*::*HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY8738	*MAT***a** *SWI2-Myc*::*TRP1 gcn5*::*HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY9391	*MAT***a** *SWI2-Myc*::*TRP1 swi5*::*hisG-URA3-hisG sin3*::*ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY9393	*MAT***a** *SWI2-Myc*::*TRP1 sin3*::*ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY9395	*MAT***a** *SWI2-Myc*::*TRP1 swi5*::*hisG-URA3-hisG ade2 can1 his3 leu2 trp1 ura3*
DY9709	*MAT***a** *swi2-E834K spt3*::*HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY9711	*MAT***a** *swi2-E834K ash1*::*LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY9715	*MAT***a** *swi2-E834K spt3*::*HIS3 ash1*::*LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY9726	*MAT***a** *swi2-E834K ade2 can1 his3 leu2 trp1 ura3*
DY9754	*MAT*α *SWI2-Myc*::*TRP1 gcn5*::*HIS3 URA3*::*GCN5*(*E173Q*) *ade2 can1 his3 leu2 trp1*
DY9791	*MAT***a** *ho*::*GFP-NLS-PEST*::*HIS3MX6 ade2 can1 his3 leu2 trp1 ura3*
DY9862	*MAT***a** *ho*::*GFP-NLS-PEST*::*HIS3MX6 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY9864	*MAT***a** *ho*::*GFP-NLS-PEST*::*HIS3MX6 ash1*::*LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY9865	*MAT***a** *ho*::*GFP-NLS-PEST*::*HIS3MX6 gcn5*::*TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY9868	*MAT***a** *ho*::*GFP-NLS-PEST*::*HIS3MX6 gcn5*::*TRP1 ash1*::*LEU2 ade2 can1 his3 leu2 lys2 trp1 ura3*
DY9923	*MAT***a** *SWI2-Myc*::*TRP1 sin3*::*ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY9927	*MAT***a** *SWI2-Myc*::*TRP1 gcn5*::*HIS3 sin3*::*ADE2 ade2 can1 his3 leu2 trp1 ura3*
DY10009	*MAT***a** *swi2-E834K spt15*::*LEU2* + *SPT15*(YCp-*URA3-ADE3*) *ade2 ade3 can1 his3 leu2 trp1 ura3*
DY10145	*MAT***a** *swi2-314 sin3*::*ADE2 ade2 can1 his3 leu2 trp1 ura3*

stable promoter occupancy by SWI/SNF requires both its bromodomain and histone acetylation (39).

Histone acetylation is a dynamic process in vivo, with histone deacetylase (HDAC) enzymes counteracting the activities of histone acetyltransferases. The yeast *SIN3* gene was first identified as a suppressor mutation that allows *HO* expression in the absence of the Swi5 activator (65, 78). Sin3 exists in a complex with the Rpd3 histone deacetylase (46, 47), and genetic experiments have shown that *sin3* and *rpd3* mutations have similar phenotypes (80). We previously examined the abilities of suppressor mutations to allow *HO* expression in the absence of various activators (85, 86). A *sin3* mutation suppresses the requirement for the Swi5 DNA-binding protein or the Gcn5 HAT, but *sin3* does not allow *HO* expression in the absence of Swi2 (in the SWI/SNF remodeler) or Swi6 (in the SBF DNA-binding protein).

Our previous genetic analysis demonstrated that some mutations can suppress temporally downstream events, such as the lack of the SBF (Swi4/Swi6) factor, but not upstream factors, such as mutations in SWI/SNF (86). These results suggest that the regulatory pathway is not simply linear. In this study, we have used chromatin immunoprecipitation assays and genetic analysis to dissect the complex regulation of this promoter. Our results show that SAGA and SWI/SNF bindings are interdependent. We show that the *ash1* mutation suppresses a *gcn5* mutation with respect to both *HO* expression and SWI/SNF binding, thereby revealing a new role for Gcn5 in overcoming Ash1 repression in mother cells. Histone acetylation by Gcn5 is required for SWI/SNF binding at *HO*, and thus, the Ash1 repressor protein apparently functions to inhibit SWI/SNF binding to *HO*. Our genetic analysis of a hypomorphic *swi2* allele suggests that SWI/SNF facilitates TATA-binding protein (TBP) binding, either directly or indirectly.

MATERIALS AND METHODS

All yeast strains used are listed in Table 1 and are isogenic in the W303 background (81). Standard genetic methods were used for strain construction (74). The plasmids used are listed in Table 2. The cells were grown at 30°C in yeast extract-peptone-dextrose medium or, to select for plasmids, synthetic complete medium with 2% glucose supplemented with adenine, uracil, and amino acids as appropriate but lacking essential components (74). For synchrony ex-

TABLE 2. Plasmid list

Plasmid	Description	Source or reference
YEplac112	YEp-*TRP1* vector	36
pSVA12	GFP plasmid	59
pSVA13	GFP-Cln2 PEST fusion plasmid	59
M4916	GFP-NLS fusion plasmid	This work
M4914	6.2-kb *HO* fragment in YEp-*TRP1* plasmid	This work
M4917	*HO*::GFP-NLS in YEp-*TRP1* plasmid	This work
M4951	*HO*::GFP-NLS-Cln2 PEST in YEp-*TRP1* plasmid	This work
pRS314	YCp-*TRP1* vector	75
pTM8	TBP(wild type) in YCp-*TRP1* plasmid	51
M4909	TBP(V71E) in YCp-*TRP1* plasmid	This work
M4956	TBP(N159K) in YCp-*TRP1* plasmid	This work
M4827	TBP(wild type) in YEp-*TRP1* plasmid	13
pKA92	TBP(V71A) in YCp-*TRP1* plasmid	4
pKA103	TBP(N159D) in YCp-*TRP1* plasmid	4
spt15-301	TBP(L114F) in YCp-*TRP1* plasmid	5
TBP(N159L)	TBP(N159L) in YCp-*TRP1* plasmid	51
TBP(V161A)	TBP(V161A) in YCp-*TRP1* plasmid	51
pDE58-1	TBP(V161A) in YCp-*TRP1* plasmid	31
pUN45-IID (K138L)	TBP(K138L) in YCp-*TRP1* plasmid	18
pUN45-IID (K145L)	TBP(K145L) in YCp-*TRP1* plasmid	18
pRS316	YCp-*URA3* vector	75
pRS416-*SWI2*	*SWI2* in YCp-*URA3* plasmid	40
YEp24	YEp-*URA3* vector	15
pLN138-4	*SWI2* in YEp-*URA3* plasmid	1

periments by the α-factor arrest-and-release method (17), cells were grown in YM-1 medium (38) at 25°C and arrested by incubation with 3 μM α-factor (University of Utah Peptide Synthesis Core Facility). The cells were monitored by light microscopy to determine when they were arrested, typically for 2 to 2.5 h as determined by microscopy. After arrest, the cells were filtered, washed with 1 volume of YM-1 medium, and released into fresh YM-1 medium containing 0.15 mg/ml pronase (Sigma; 81748) at 25°C. The *gcn5 ash1* strain required 15 μM α-factor for efficient arrest. Fluorescence-activated cell sorter analysis and budding indices showed that the cells were in synchrony.

The genomic *HO* locus was modified to replace the open reading frame with green fluorescent protein (GFP)-nuclear localization signal (NLS)-PEST to allow visualization of *HO* expression by GFP fluorescence in mother and daughter cells. An EcoRI/XbaI fragment containing the *HO* open reading frame with 3.5 kb upstream and 0.8 kb downstream of flanking DNA was first cloned into YEplac112 (36) to make plasmid M4914. Oligonucleotides F1297 and F1298, which encode the T antigen NLS (Pro-Lys-Lys-Lys-Arg-Lys-Val) and have BsrGI and AscI overhangs, were annealed and cloned into the GFP plasmid pSVA12 (59) that had been cleaved with BsrGI and AscI, generating plasmid M4916 (GFP-NLS fusion plasmid). The GFP-NLS was then amplified with primers F1304 and 1305 that had extensions with homology to *HO*, and then the PCR product and BssHII-cleaved plasmid M4914 were cotransformed into strain DY7158 (*hmr*Δ::*URA3*) to generate M4917 (*HO*::*GFP-NLS*) by homologous recombination. The PEST sequence from *CLN2* was amplified from pSVA13 (59) using primers F1339 and F1340 that targeted the PEST in frame at the 3′ end of *HO*::*GFP-NLS* by homologous recombination with AscI/PmeI-digested M4917 in strain DY150, generating plasmid M4951. M4951 was then digested with SalI and integrated at the *HO* locus in strain DY150 to replace the *HO* open reading frame with GFP-NLS-PEST, generating strain DY9791. Proper integration was confirmed by Southern analysis.

For fluorescence microscopy, cells were synchronized by α-factor arrest and release as described above, except that the YM-1 medium was supplemented with 0.025% adenine. Fluorescence was monitored in G_1 of the second cell cycle following release, 2 to 2.5 h following release. Pictures were taken with an Olympus BX51 fluorescence/DIC microscope and a MagnaFire SP S99810 camera.

RNA levels were determined with S1 nuclease protection assays using *HO* and *CMD1* probes as described previously (11). Chromatin immunoprecipitations were performed as described previously (12) using 9E11 monoclonal antibody (Abcam) to the Myc epitope and antibody-coated magnetic beads (Pan Mouse IgG beads; Dynal Biotech). Real-time PCR and calculations were performed as described previously (32). The following PCR primers were used: *HO* URS1, F1093 (TATACCCAATCGCTGCGTGC) and F1094 (AGCCGCCACGAATC AAACTT); *HO* URS2, F1095 (GGCAAACCTAATGTGACCGT) and F1096 (ACAGGACTTGCGAACCCTTT); *HO* TATA "A," F1101 (GCTGGGCGTT ATTAGGTGTG) and F1102 (GAGTTAGCCGTGACGTTTGC); *HO* TATA "B," F1154 (CCATATCCTCATAAGCAGCA) and F1155 (AAGCTCTGTGT TTGGTTTTT); *CLN2*, F996 (GTTATCAATTCATGCGCGCT) and F997 (A GATCAACATTTCGCAGGTT); and *YDL224c* (control), F798 (CTCGAACC GGTAGTTTTACA) and F799 (GAGAAACCTTAAGCGTTATT).

For the ChIP-HindIII digestion experiment, the same methods were used for formaldehyde cross-linking, chromatin shearing by sonication, and immunoprecipitation. Magnetic Dynabeads (Dynal Biotech) with the immunoprecipitated chromatin were resuspended in 200 μl of 1× HindIII reaction buffer and split into two aliquots of 100 μl each. One aliquot was digested with 50 units of HindIII restriction enzyme, while the other portion was incubated with only buffer; both samples were rotated overnight at 37°C. The beads were then washed to remove DNA fragments that were nonspecifically bound, followed by elution of the DNA from the beads, reversal of the cross-links, purification of DNA, and analysis by real-time PCR.

RESULTS

Increased acetylation allows SWI/SNF binding without the Swi5 activator. It is believed that SWI/SNF is recruited to *HO* URS1 by the Swi5 activator. A direct interaction between Swi5 and SWI/SNF has been demonstrated in vitro (66), and a *swi5* mutation eliminates SWI/SNF binding to *HO* in vivo (25). Sin3 is a component of a histone deacetylase complex (45, 47), and a *sin3* mutation allows *HO* expression in the absence of Swi5 (65). This result raises several questions. Is SWI/SNF required for *HO* expression in the *sin3 swi5* double mutant? Is the SWI/SNF coactivator recruited to the *HO* promoter in the *sin3 swi5* double mutant?

Isogenic strains were constructed, differing at the *SWI5*, *SWI2*, and *SIN3* loci, and an S1 nuclease assay was performed to measure *HO* mRNA levels in logarithmically growing cells (Fig. 1B). The *swi2* gene disruption eliminates the catalytic subunit of SWI/SNF. Mutations in *SWI5* or *SWI2* result in a drastic reduction in *HO* mRNA levels (lanes 2 to 4), while a *sin3* mutation allows *HO* to be expressed in the absence of the Swi5 activator (lane 6). A *sin3* mutation does not, however, allow strong *HO* expression in either the *swi2* or the *swi5 swi2* mutant strain (lanes 7 and 8). We conclude that SWI/SNF is required to achieve significant *HO* expression in the *sin3 swi5* double mutant.

ChIP experiments were performed using Swi2-Myc epitope-tagged strains to determine whether SWI/SNF binds to the *HO* promoter in the *sin3 swi5* double mutant in which *HO* is expressed (Fig. 1C). Swi2 binding to *HO* is detected in the wild type, but this binding is abolished in the *swi5* mutant, as previously described (25). Importantly, Swi2 binding to *HO* is restored in the *swi5 sin3* mutant. Similar results are seen at the both the URS1 and URS2 regions of the promoter. We also examined binding of Swi2 to the *HO* promoter in *swi5 sin3* mutant cells that had been synchronized in the cell cycle by α-factor arrest and release. SWI/SNF binding to *HO* in wild-type cells is periodic and occurs in the second cell cycle following release, at a time coincident with *HO* expression (for an example, see Fig. 5A). We found that in the *swi5 sin3* mutant, *HO* is expressed in both the first and second cycles after release, as previously described (65). In the *swi5 sin3* mutant, SWI/SNF binding is periodic and correlates with *HO* expres-

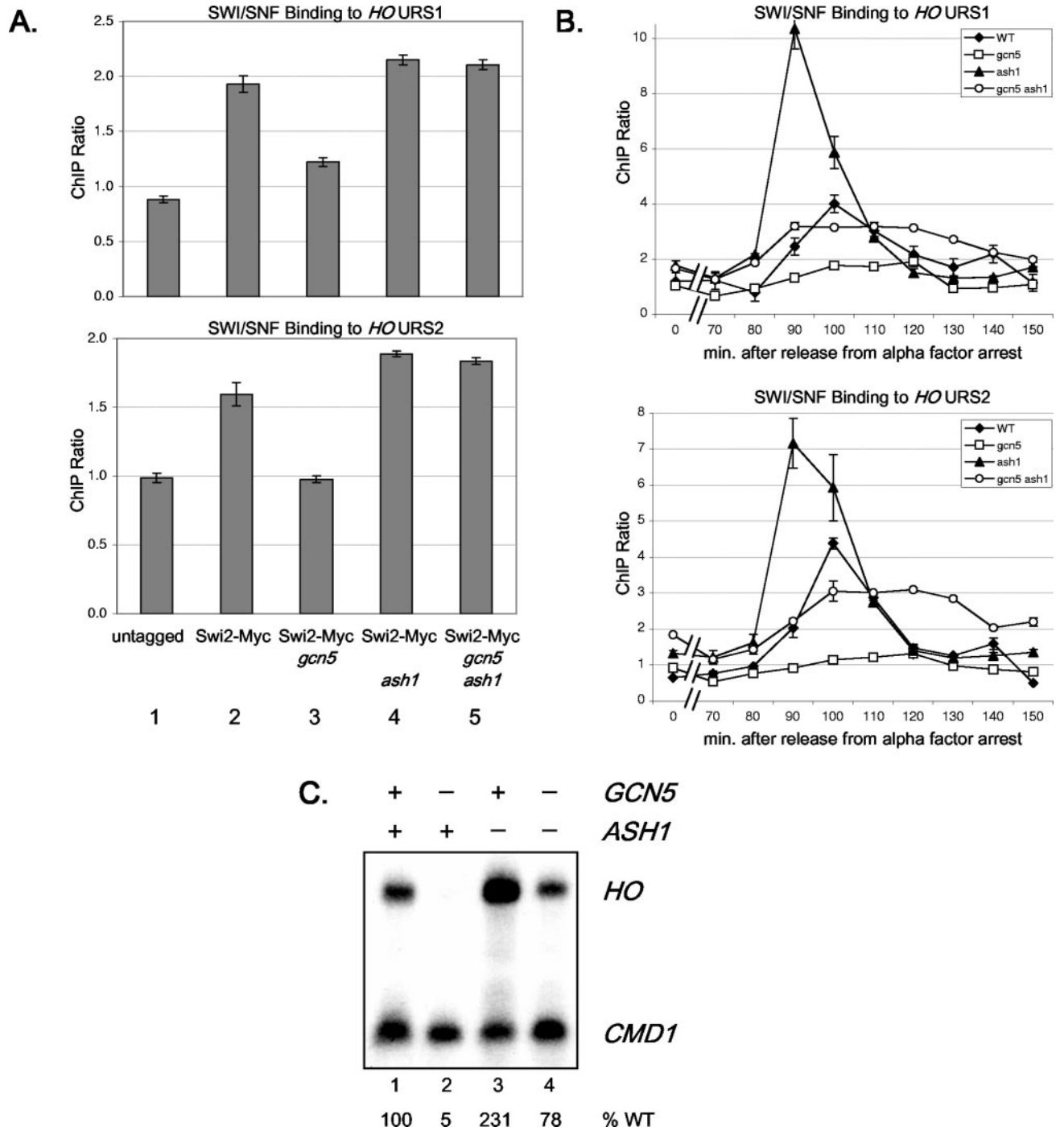


FIG. 2. Ash1 overcomes the *gcn5* requirement for SWI/SNF binding and *HO* expression. (A) SWI/SNF binding to *HO* URS1 and URS2 is restored in a *gcn5 ash1* mutant. ChIP was performed with an untagged strain (DY150) and with Swi2-Myc strains that were wild type (DY6151), *gcn5* (DY8738), *ash1* (DY7403), or *gcn5 ash1* (DY8736). SWI/SNF binding to *HO* URS1 and URS2 was measured by real-time PCR, and the units are arbitrary after normalization to a *YDL224c* internal control. The error bars show the standard deviations of the ChIP PCRs performed in triplicate. (B) SWI/SNF binding to *HO* URS1 and URS2 during the cell cycle. Swi2-Myc-tagged strains DY6151 (wild type), DY8738 (*gcn5*), DY7403 (*ash1*), and DY8736 (*gcn5 ash1*) were synchronized by α-factor arrest and release, and samples were taken at various times for ChIP. SWI/SNF binding to *HO* URS1 and URS2 was measured by real-time PCR, and the units are arbitrary after normalization to a *YDL224c* internal control. The error bars show the standard deviations of the ChIP PCRs performed in triplicate. (C) RNAs were prepared from strains DY150 (wild type), DY5265 (*gcn5*), DY4394 (*ash1*), and DY5268 (*gcn5 ash1*) and used for S1 nuclease protection assays to measure *HO* and *CMD1* (internal control) RNA levels.

sion (data not shown). It has been shown that a *sin3* mutation results in increased histone acetylation of the *HO* promoter throughout the cell cycle (53), and it is possible that such acetylation facilitates SWI/SNF binding to the promoter.

Suppression of *gcn5* results in both SWI/SNF binding and *HO* expression. A *sin3* mutation also allows *HO* to be expressed despite a *gcn5* mutation (86) and raises the question of whether SWI/SNF binds to the *HO* promoter in the *gcn5 sin3*

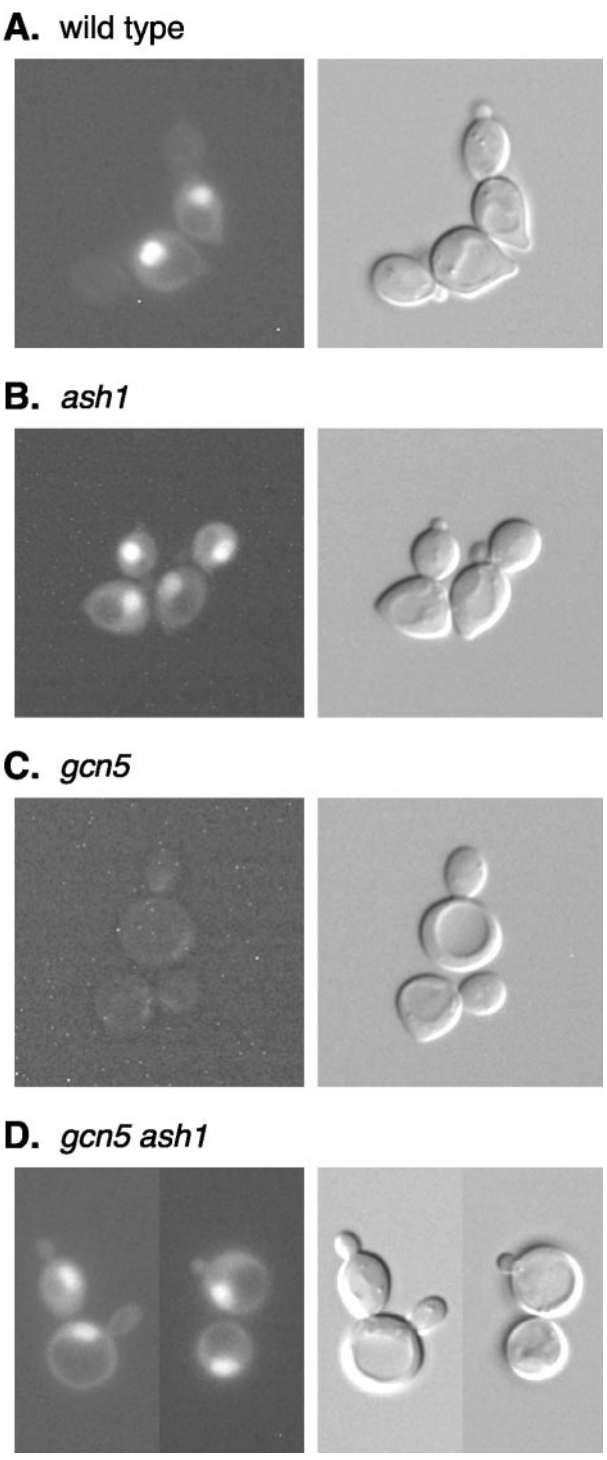


FIG. 3. *HO* expression in mothers and daughters in a *gcn5 ash1* strain. Strains were arrested with α-factor to synchronize in the cell cycle, and samples were collected between 2 and 2.5 hours following release. Fluorescent images (left) and corresponding DIC images (right) are shown. The following strains were used: (A) DY9862 (*HO*::*GFP-NLS-PEST*), (B) DY9864 (*HO*::*GFP-NLS-PEST ash1*), (C) DY9865 (*HO*::*GFP-NLS-PEST gcn5*), and (D) DY9868 (*HO*::*GFP-NLS-PEST gcn5 ash1*). The α-factor arrest results in changed cell shape, marking mother cells. This shape change is not as visible in the *gcn5 ash1* strain, possibly because the strain arrested poorly with α-factor.

strain. We measured Swi2-Myc binding to *HO* in isogenic strains differing at *GCN5* and *SIN3* using ChIP assays (Fig. 1D). There is reduced binding to both *HO* URS1 and URS2 in the *gcn5* strain (bar 3), especially at URS2. These results differ from those of Cosma et al. (25), as discussed below. Importantly, SWI/SNF binding is restored in the *gcn5 sin3* double mutant (bar 5), where *HO* is expressed. The effect of the *gcn5* mutation is more pronounced at URS2 than at URS1, consistent with previous experiments showing that histone acetylation at *HO* in the G_1 phase of the cell cycle is restricted to a 1-kb region encompassing the TATA region and the SBF binding sites in URS2 (53). These experiments show a correlation between *HO* expression and SWI/SNF binding in the *gcn5 sin3* mutant strain.

Acetylation by Gcn5 is needed for sustained binding of SWI/SNF to *HO*. We found that a *gcn5* mutation sharply reduced Swi2 binding at URS1 and completely eliminated Swi2 binding at URS2 (Fig. 2A, bar 3). This result appears to be discrepant with that of Cosma et al. (25), who reported that SWI/SNF binding to URS2 was unaffected by a *gcn5* mutation. (Cosma et al. did not report the effect of a *gcn5* mutation on SWI/SNF binding to URS1.) However, there is an important difference between the two sets of experiments. Their studies used a Swi2-Myc *gcn5 ash1* strain, while we used a Swi2-Myc *gcn5 ASH1*$^+$ strain. Ash1 is the daughter-specific repressor of *HO*, and most of Cosma et al.'s (25) ChIP experiments were conducted with *ash1* mutants, presumably to increase the signal by allowing factor binding in both mothers and daughters.

To investigate the role of *ASH1* in regulating SWI/SNF binding, we performed ChIP assays with isogenic strains differing at the *GCN5* and *ASH1* loci (Fig. 3A). Consistent with Cosma et al.'s (25) results, we did see a subtle increase in Swi2 binding to URS2 in an *ash1* mutant compared to the wild type (Fig. 2A, URS2, compare bar 4 to bar 2). More importantly, while Swi2 bound weakly to *HO* URS1 and not at all to *HO* URS2 in the *gcn5* mutant (Fig. 2A, bar 3), strong Swi2 binding was observed in the *gcn5 ash1* strain (Fig. 2A, bar 5). Thus, there is no discrepancy in the experiments examining binding in the *gcn5 ash1* strains. Moreover, the results suggest that Gcn5 is required for SWI/SNF binding to *HO* and that SWI/SNF binding is inhibited by Ash1. We note that Cosma et al. (25) previously suggested that Ash1 inhibits SWI/SNF binding to *HO* in daughter cells.

We next examined the association of SWI/SNF with the *HO* promoter during the cell cycle. Four strains, the wild type, *gcn5*, *ash1*, and *gcn5 ash1*, each with a Swi2-Myc epitope tag, were synchronized by an α-factor arrest-and-release protocol. Flow cytometry analysis showed that good synchrony was maintained through the time course of these experiments in the wild-type, *gcn5*, and *ash1* strains (data not shown). Samples were collected at various time points following release for RNA analysis and ChIP assays. *HO* is not expressed in the first cycle following release from an α-factor arrest (64), and thus, our analysis begins with the second cycle. *HO* mRNA, measured by S1 nuclease protection assay, peaks at 100 to 110 min following release (see Fig. 5A). Swi2 binding to URS1 and URS2 was assessed by ChIP in the second cell cycle following release (Fig. 2B). Again, Swi2 binding to *HO* was not seen in the *gcn5* mutant, while Swi2 showed cell cycle periodicity in its association with the *HO* promoter in the wild type and the *ash1*

nutant. The level of Swi2 binding was higher in the *ash1* strain than in the wild type, and the peak of binding in early G_1 preceded that seen in the wild type. Binding of Swi2 to both the URS1 and URS2 regions was observed in the *gcn5 ash1* mutant strain. Although SWI/SNF binding shows weak cell cycle periodicity in this mutant, the peak-to-trough levels are less than in *GCN5* strains. We found that the *gcn5 ash1* strain was defective for α-factor-mediated arrest, and a fivefold-larger amount of α-factor was used to arrest the mutant. The cell cycle synchrony in the *gcn5 ash1* strain was not as good as in wild type, and this might have contributed to the poor periodicity in Swi2 binding in the *gcn5 ash1* double mutant. Nonetheless, it is clear that Swi2 binding is much higher in the *gcn5 ash1* strain than in the *gcn5* single mutant.

Ash1 suppresses the *gcn5* defect in *HO* expression. Binding of SWI/SNF to the *HO* promoter is lost in a *gcn5* mutant and restored in a *gcn5 ash1* double mutant. These results suggest that Ash1 inhibits the association of SWI/SNF with the *HO* promoter and also raise the question of whether *HO* is expressed in a *gcn5 ash1* strain. An S1 nuclease protection assay was used to measure *HO* mRNA in isogenic strains differing at the *GCN5* and *ASH1* loci (Fig. 2C). A *gcn5* mutation markedly reduced *HO* expression (lane 2), and an *ash1* mutation resulted in increased *HO* expression (lane 3), presumably because both mother and daughter cells express *HO*. Importantly, *HO* expression was restored in the *gcn5 ash1* double-mutant strain (lane 4). This experiment suggests that the Gcn5 histone acetyltransferase is primarily required at *HO* to overcome repression by Ash1. However, we note that *HO* expression in the *gcn5 ash1* strain was slightly less than in the wild type, suggesting that Gcn5 has additional functions at *HO*, presumably to facilitate the binding of other factors.

***HO* is expressed in mother and daughter cells in the *gcn5 ash1* strain.** *HO* is normally expressed only in mother cells. Mutation of the *ASH1* gene encoding a daughter-specific repressor results in *HO* expression in both mothers and daughters (14, 76). As *HO* is expressed in a *gcn5 ash1* mutant (Fig. 2C), we asked how an *ash1* mutation allows *HO* expression in the absence of the Gcn5 coactivator. We have two hypotheses. In the first, *HO* expression in daughters does not require Gcn5. According to this model, daughter cells fail to express *HO* due to the presence of the Ash1 repressor but would express the gene in a *gcn5 ash1* strain because Gcn5 is not required for *HO* expression in daughters. In contrast, Gcn5 is absolutely required for *HO* expression in mothers. This model predicts that *HO* should be expressed exclusively in daughter cells in the *gcn5 ash1* double mutant.

We consider this explanation unlikely, as it proposes that *HO* expression is Gcn5 dependent in mothers but Gcn5 independent in daughters. Instead, we favor a second hypothesis, that Ash1 also represses *HO* transcription in mother cells and that Gcn5 is required to overcome this Ash1 repression. Gcn5 is unable to overcome this repression in daughters, presumably because daughters have much more Ash1 than mothers.

To address these questions, we examined *HO* expression in mother and daughter cells in a *gcn5 ash1* mutant using a fluorescent reporter. A result where *HO* is expressed only in daughter cells in the *gcn5 ash1* mutant would argue that *HO* expression in daughters does not require Gcn5. The other result, *HO* expression in both mother and daughter *gcn5 ash1* cells, would demonstrate that Ash1 represses *HO* transcription in mothers, as well as daughters.

To address this question, we modified the chromosomal *HO* locus by replacing the *HO* open reading frame with a GFP-NLS-PEST fusion protein. The NLS targets the protein to the nucleus, facilitating microscopic localization, and the PEST instability sequence from the *CLN2* gene (59) decreases the half-life of the protein and prevents perdurance of the protein to the next cell cycle. We used the approach of Sil and Herskowitz (76) to distinguish mother and daughter cells during microscopy. Cells were arrested in the cell cycle with α-factor, and the cells changed shape ("shmoo") in response to the pheromone. Following release from α-factor arrest, GFP-NLS-PEST localization during the cell cycle was monitored by fluorescence microscopy. Mother cells are shmoo shaped, and daughter cells are round, allowing clear identification of cell types while examining cells with asymmetric *HO* expression.

As shown in Fig. 3A, the *HO*::GFP-NLS-PEST reporter accumulated only in mother cell nuclei in wild-type cells. In contrast, GFP-NLS-PEST was visible in both mother and daughter cells in the *ash1* mutant (Fig. 3B) but was not visible in *gcn5* cells (Fig. 3C). Importantly, GFP-NLS-PEST was present in both mother and daughter cell nuclei in the *gcn5 ash1* double-mutant strain (Fig. 3D). This demonstrates that Gcn5 is required to overcome Ash1 repression in mother cells.

***sin3* and *ash1* are additive in suppression of the *gcn5* mutation at *HO*.** Recent work has shed light on the mechanism of Ash1 repression of the *HO* promoter. It has been demonstrated that there are two Sin3-Rpd3 HDAC complexes, a small 0.6-MDa complex and a large 1.2-MDa complex (20, 48). The two complexes have Sin3, Rpd3, and Ume1 as shared subunits, while the small and large complexes each contain unique subunits. The large Sin3-Rpd3 complex contains the sequence-specific repressors Ash1 and Ume6, which are suggested to play roles in targeting the complex to specific promoters (19). Recruitment of the large Sin3-Rpd3 HDAC complex to the *HO* promoter by Ash1 provides a mechanism of transcriptional repression by Ash1. Importantly, the defect in *HO* expression in a *gcn5* mutant can be suppressed by mutations in *ASH1* (Fig. 2C), *RPD3*, or *SIN3* (86). It follows from this model of Ash1 recruiting the large Sin3-Rpd3 complex to the promoter that the suppression of *gcn5* provided by the *sin3* and *ash1* mutants should not be additive. We measured *HO* RNA from isogenic strains differing at *GCN5*, *SIN3*, and *ASH1* (Fig. 4A). Consistent with previous observations, the *gcn5* mutant showed a strong defect in *HO* expression that was suppressed by the *sin3* and *ash1* mutations (lanes 5, 6, and 7). Surprisingly, we found that the suppression in the *gcn5 sin3 ash1* triple mutant (lane 8) was significantly higher than in either double mutant (lanes 6 and 7), and the level of *HO* RNA in this triple mutant was similar to that in the wild type. This observation suggests that Ash1 may exert its repressive role at *HO* both within the Sin3-Rpd3 complex and also independently of it. Additionally, Sin3-Rpd3 may influence regulation of the *HO* promoter via its untargeted global role or via its recruitment to the promoter by transient interactions with other factors at the promoter (20).

Gcn5 enzymatic activity is required for SWI/SNF binding and *HO* expression. Our experiments showing that Gcn5 is required for sustained binding of SWI/SNF to the *HO* pro-

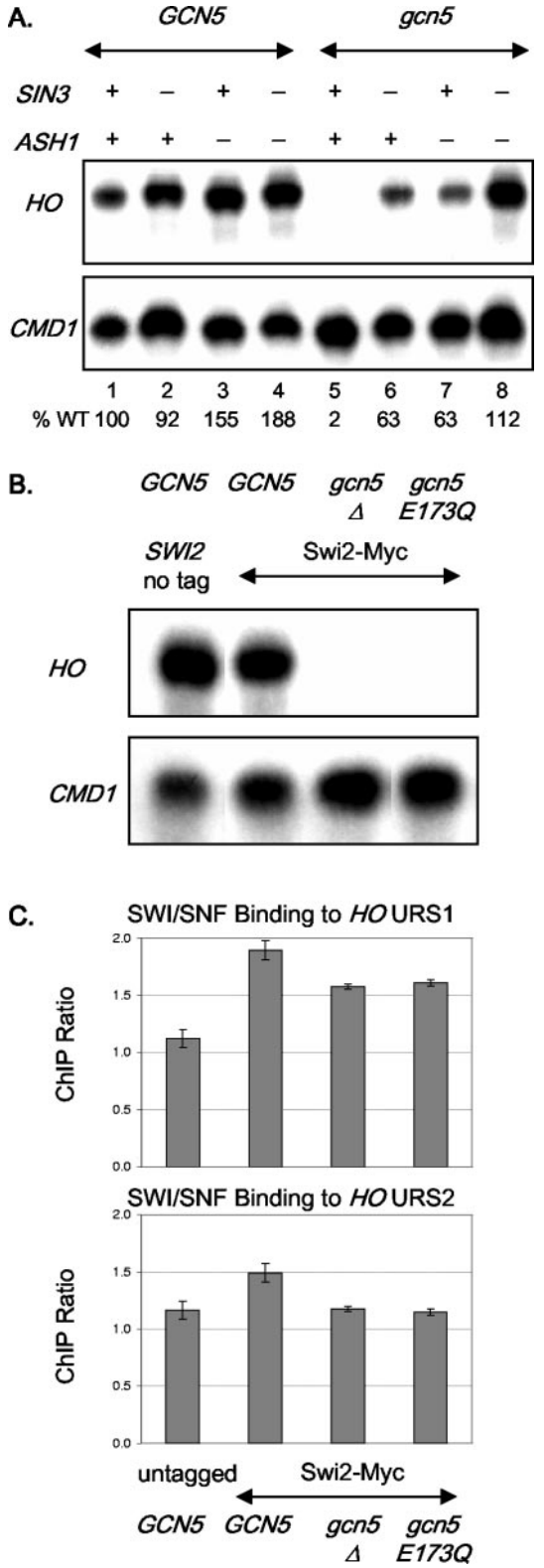
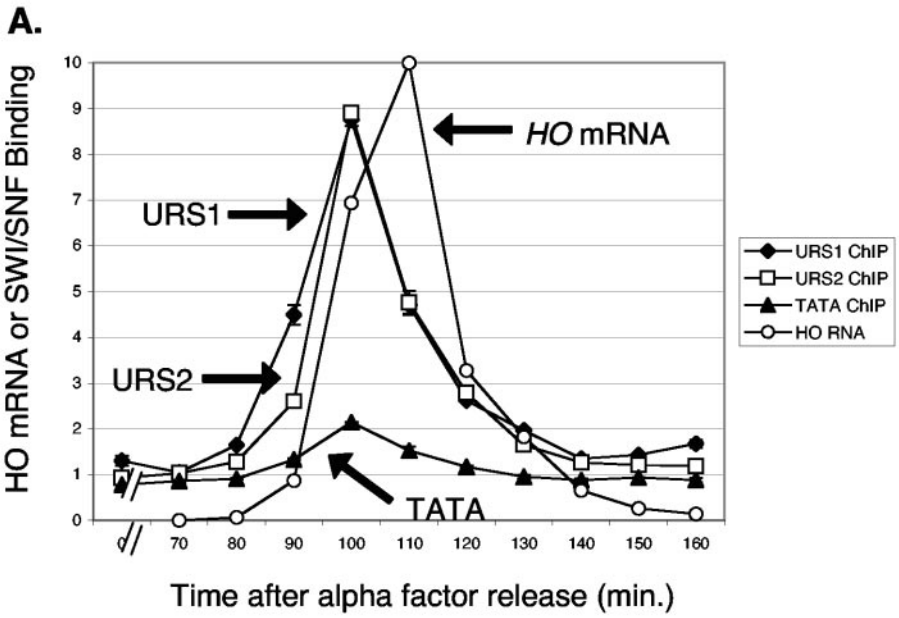


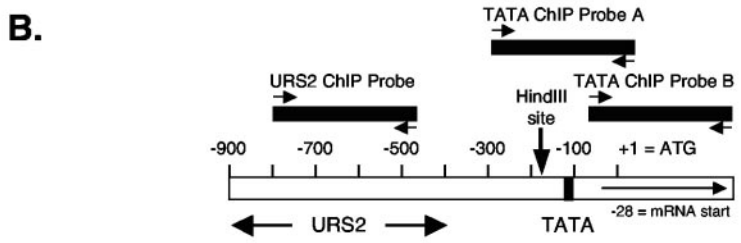
FIG. 4. Gcn5 catalytic activity is necessary for sustained SWI/SNF binding. (A) *sin3* and *ash1* are additive in suppressing *gcn5*. RNAs were prepared from strains DY150 (wild type), DY984 (*sin3*), DY4394 (*ash1*), DY6394 (*sin3 ash1*), DY5265 (*gcn5*), DY5297 (*gcn5 sin3*), DY7387 (*gcn5 ash1*), and DY7385 (*gcn5 sin3 ash1*) and used for S1 nuclease protection assays to measure *HO* and *CMD1* (internal control) RNA levels. (B) Gcn5 catalytic activity is necessary for *HO* expression. RNAs were prepared from strains DY150 (no tag), DY6151 (Swi2-Myc), DY8738 (Swi2-Myc *gcn5*Δ), and DY9754 (Swi2-Myc *gcn5-E173Q*) and used for S1 nuclease protection assays to measure *HO* and *CMD1* (internal control) RNA levels. (C) Gcn5 catalytic activity is necessary for sustained SWI/SNF binding. ChIP was performed with an untagged strain (DY150) and with Swi2-Myc strains that were wild type (DY6151), *gcn5*Δ (DY8738), and *gcn5-E173Q* (DY9754). SWI/SNF binding to either *HO* URS1 or URS2 was measured by real-time PCR, and the units are arbitrary after normalization to a *YDL224c* internal control. The error bars show the standard deviations of the ChIP PCRs performed in triplicate.

moter used strains with a *gcn5* gene disruption. It is possible that the histone acetyltransferase activity of Gcn5 is not required but that the mere presence of the Gcn5 polypeptide as a component of SAGA is sufficient for association of SWI/SNF with the *HO* promoter. An E173Q mutation in Gcn5 eliminates the histone acetyltransferase catalytic activity (82), and we used this mutant allele to address this question. As shown in Fig. 4B, *HO* expression is eliminated in strains with either a *gcn5* gene disruption or a *gcn5*(E173Q) mutation. ChIP experiments show that Swi2 binding to URS1 and URS2 is similarly affected by a *gcn5* gene disruption and by the *gcn5*(E173Q) mutation (Fig. 4C). We note that the *gcn5* gene disruption has a more modest reduction in SWI/SNF binding at URS1 in this experiment than is shown in Fig. 1 and 2. We conclude that a point mutation eliminating the Gcn5 histone acetyltransferase activity blocks association of SWI/SNF with the *HO* promoter.

SWI/SNF binding is detected at the *HO* TATA region. Previous studies have shown that *HO* expression is dependent on the SWI/SNF chromatin-remodeling complex, and ChIP experiments have shown SWI/SNF binding to both the URS1 and URS2 regions of the *HO* promoter. We have shown genetic interactions between *SWI2* and the TBP and TFIIA basal transcription factors (13). Moreover, our ChIP experiments showed that TBP may be the last factor to bind to *HO* before transcription and that mutations that suppress activator defects also result in prolonged TBP binding at *HO* (85). These results lead to a model in which chromatin remodeling by SWI/SNF facilitates TBP binding to the *HO* promoter. Therefore, we used ChIP assays to investigate the presence of SWI/SNF at the *HO* TATA region.

We examined the association of SWI/SNF to the *HO* promoter during the cell cycle. Cells with a Swi2-Myc epitope tag were synchronized by α-factor arrest and release, and Swi2 binding to URS1 and URS2 was assessed by ChIP in the second cell cycle following release (Fig. 5A). Our ChIP assays showed SWI/SNF binding to both URS1 and URS2, with the peak of binding at 100 min after release. However, our experiments detected SWI/SNF binding to the URS1 and URS2 regions of *HO* at essentially the same time, while Cosma et al. (25) reported that SWI/SNF is present at URS1 before it is associated with URS2. There were methodological differences in these studies; we synchronized *ASH1* cells with an α-factor arrest-and-release protocol, while they used a *GAL*::*CDC20 ash1* strain to release cells from the M-phase arrest caused by withdrawal of Cdc20. Finally, significant SWI/SNF binding to the *HO* TATA region was also observed, coincident





		URS2 PCR product	TATA PCR product A	TATA PCR product B
Swi2-Myc tagged strain	undigested	8.9 ± 0.01	**2.9** ± 0.01	**2.2** ± 0.01
	HindIII	8.6 ± 0.01	**1.5** ± 0.01	**2.2** ± 0.01
strain without Swi2-Myc tag	undigested	1.1 ± 0.01	1.1 ± 0.01	0.8 ± 0.01
	HindIII	1.2 ± 0.01	1.1 ± 0.01	0.8 ± 0.01

FIG. 5. SWI/SNF binds to the *HO* TATA. (A) Strain DY6151 (Swi2-Myc) was synchronized by α-factor arrest and release, and samples were taken at various times for ChIP and mRNA analysis. SWI/SNF binding to the URS1, URS2, or TATA region of the *HO* promoter was measured by real-time PCR, and the units are arbitrary after normalization to a *YDL224c* internal control. The error bars show the standard deviations of the ChIP PCRs performed in triplicate. *HO* mRNA was measured by S1 nuclease protection, quantitated by phosphorimager, and normalized to *CMD1* levels (loading control). (B) Map showing relative positions of PCR primers used. Strains DY6151 (Swi2-Myc) and DY150 (untagged) were synchronized by α-factor arrest and release, and samples were taken at 90, 100, and 110 min after release for ChIP. The data shown are for the 100-min time point, but similar results were obtained with the other samples. The samples labeled HindIII were digested with HindIII after the immunoprecipitation step.

with *HO* mRNA expression and with SWI/SNF binding at URS1 and URS2.

The *HO* TATA is reasonably close to the URS2 region, where SWI/SNF binds. Thus, we considered the possibility that SWI/SNF does not actually bind to the TATA but that sufficiently large DNA fragments remain, even after shearing, that contain both the TATA region and Swi2 bound to the right end of URS2. This "spillover" from URS2 could give an artifactual ChIP signal at TATA. To rule out this artifact, we took advantage of a HindIII site present between URS2 and TATA. ChIPs were performed with two strains, Swi2-Myc and an untagged control, that had been synchronized in the cell cycle. After formaldehyde cross-linking, chromatin shearing by sonication, and immunoprecipitation, the beads with the immunoprecipitated chromatin were split in half. One part was digested with HindIII restriction enzyme, while the other portion was incubated only with buffer, followed by extensive washing to release newly cleaved and unbound fragments. The samples were then treated to reverse the cross-links and analyzed by PCR. As shown in Fig. 5B, the HindIII digestion did not significantly reduce the ChIP signal for the URS2 interval, as expected, since this region lacks HindIII sites. In contrast, the ChIP signal for the TATA ChIP probe A is largely eliminated by HindIII digestion. TATA ChIP probe A is centered over the TATA region and contains a HindIII site. Importantly, this control shows that HindIII digestion was largely effective on the immunoprecipitated DNA. Finally, we used TATA ChIP probe B, which is slightly downstream of the TATA. The ChIP

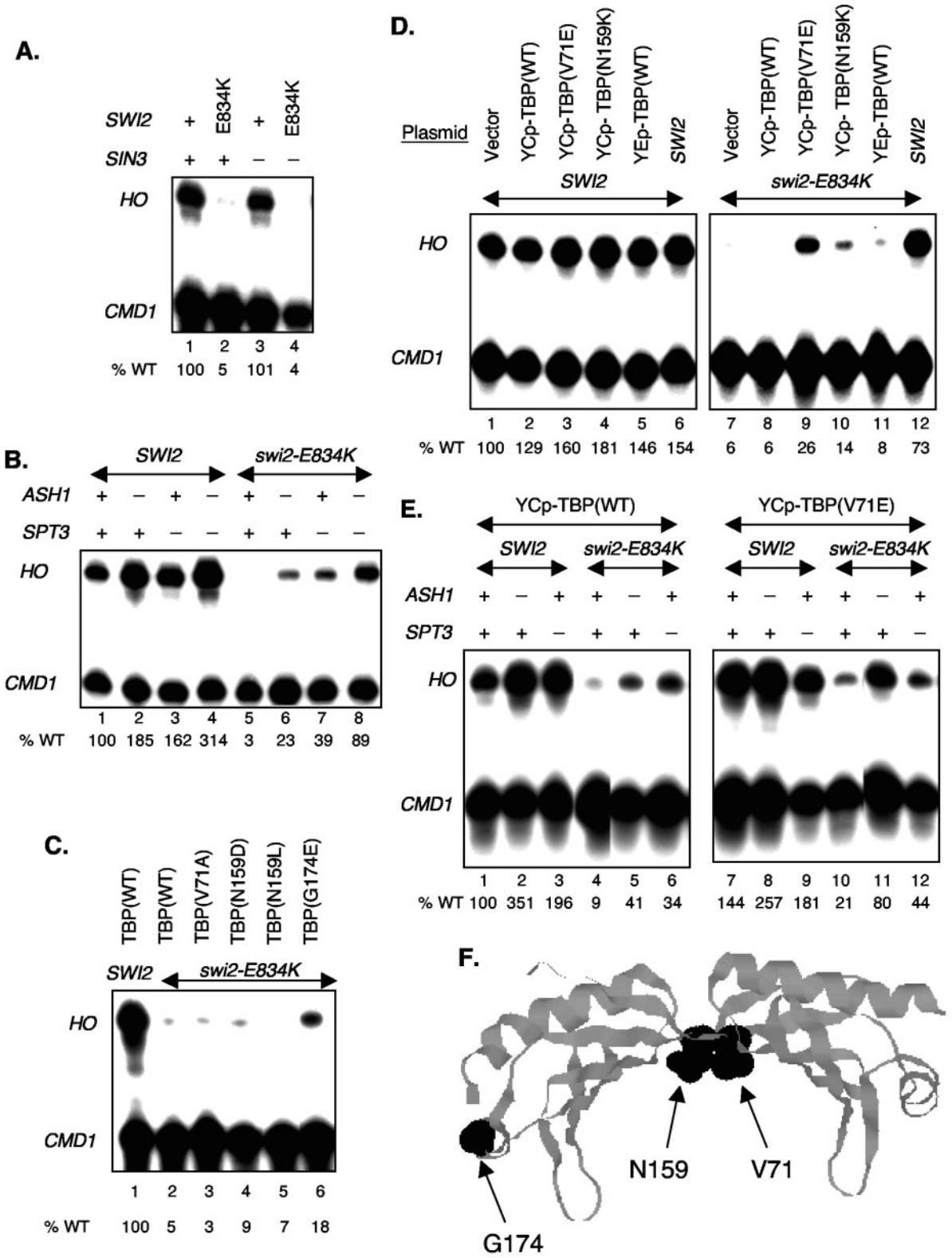


FIG. 6. Genetic suppression shows that SWI/SNF acts at the *HO* TATA. (A) RNAs were prepared from strains DY150 (wild type), DY9726 (*swi2-E834K*), DY9846 (*sin3*), and DY10145 (*swi2-E834K sin3*) and used for S1 nuclease protection assays to measure *HO* and *CMD1* (internal control) RNA levels. (B) RNAs were prepared from strains DY150 (wild type), DY4394 (*ash1*), DY6806 (*spt3*), DY7131 (*ash1 spt3*), DY9726 (*swi2-E834K*), DY9711 (*swi2-E834K ash1*), DY9709 (*swi2-E834K spt3*), and DY9715 (*swi2-E834K ash1 spt3*) and used for S1 nuclease protection assays to measure *HO* and *CMD1* (internal control) RNA levels. (C) YCp-TRP1 plasmids with either wild-type TBP or the indicated TBP mutants were transformed into DY7242 [*SWI2 spt15*Δ YCp-*URA3*-TBP(wild type)] or DY10009 [*swi2-E834K spt15*Δ YCp-*URA3*-TBP(wild type)] and then

signal for TATA ChIP probe B was completely unaffected by HindIII digestion, demonstrating that there is no "spillover" problem.

These experiments suggest that SWI/SNF binds to the *HO* TATA. We note that the binding of SWI/SNF to the *HO* TATA is modest in comparison to the binding at URS1 and URS2, and it may be that SWI/SNF binding at URS1 and URS2 is more stable than the transient binding at TATA. Nonetheless, this binding at TATA may be important for gene activation (see Discussion).

Suppression of a partially defective *swi2* allele. In order to understand the role of the SWI/SNF complex at the *HO* promoter, we investigated the genetic interactions of *swi2* with negative regulators. Previous experiments had failed to detect significant suppression of the *swi2* null allele in terms of *HO* expression (86), and thus, we decided to use a partially defective *swi2* allele. The *swi2-314* allele was isolated in a screen for mutations that reduced expression of an *HO-lacZ* reporter (16). While a *swi2* gene disruption results in a severe growth defect, a strain with the *swi2-314* allele shows only a mild growth defect (data not shown). Through DNA sequencing, we have determined that *swi2-314* contains an E834K mutation. Swi2 is the catalytic subunit of the SWI/SNF chromatin-remodeling complex and belongs to a class of nucleic-acid-stimulated ATPases and DNA helicases that share seven sequence motifs (72). The E834K residue is close to the motif Ia and is highly conserved from fungi to *Drosophila* Brm and human BRG1. Western immunoblotting shows that Swi2(E834K) is stable, accumulating at levels similar to those of wild-type Swi2 (data not shown). We conclude that *swi2-314* encodes a partially functional Swi2(E834K) protein.

We decided to investigate whether mutations in negative regulators at *HO* can suppress the *swi2-E834K* mutation. As shown in Fig. 6A (lane 2), the *swi2-E834K* mutation markedly reduced *HO* expression. However, a *sin3* mutation eliminating the Sin3-Rpd3 HDAC complexes did not suppress *swi2-E834K* (Fig. 6A, lane 4). The Spt3 component of the SAGA complex acted negatively at *HO*, as an *spt3* mutation allowed *HO* expression despite a *gcn5* mutation (85). We therefore examined *HO* expression in a *swi2-E834K spt3* strain and determined that *spt3* partially suppressed the defect in *HO* expression caused by *swi2-E834K* (Fig. 6B, compare lanes 5 and 7). Similarly, *ash1* provided partial suppression, as seen in the *swi2-E834K ash1* strain (lane 6). Combining the two suppressor mutations resulted in the strongest suppression, as seen in the *swi2-E834K ash1 spt3* triple-mutant strain (lane 8). Several conclusions can be made from this experiment. First, the fact that an *spt3* mutation allowed *HO* expression despite a defective SWI/SNF suggests that Spt3 and SWI/SNF act in opposition. Second, the suppression of *swi2-E834K* by an *ash1* mutation supports the idea that Ash1 inhibits SWI/SNF binding to *HO*. Finally, the fact that *spt3* and *ash1* showed additive effects suggests that Spt3 and Ash1 function in independent pathways to inhibit *HO* expression.

Spt3 is known to physically and genetically interact with TBP (31), and an *spt3* mutation causes an increase in TBP binding at *HO* (85). Genetic experiments with the TBP(G174E) substitution have shown allele-specific interactions with *SPT3* (31), and the TBP(G174E) substitution is thought to reduce interaction with Spt3. Interestingly, TBP(G174E) also allowed *HO* expression in strains with a *gcn5* null mutation (85). As the *spt3* null mutation suppressed the *HO* transcription defect of *swi2-E834K*, we reasoned that the TBP(G174E) allele should show a similar effect. A plasmid shuffle was used to introduce YCp plasmids with wild-type or mutant TBP into a *swi2-E834K* strain, and *HO* mRNA was measured in these strains. Partial suppression of the *HO* expression defect of *swi2-E834K* was observed in the presence of the TBP(G174E) mutant (Fig. 6C, compare lanes 2 and 6). Thus, the TBP(G174E) substitution permits *HO* expression despite the *swi2-E834K* substitution.

The results described above indicate that a TBP mutation can suppress the *HO* expression defect caused by *swi2-E834K*. We recently identified dominant gain-of-function mutations in *SPT15*, the gene encoding TBP, that allow *HO* expression in the absence of specific activators. The isolation of these mutants will be described elsewhere (J. W. Landon and D. J. Stillman, unpublished data). We asked if these dominant TBP mutants would allow *HO* expression in *swi2* mutant strains. *HO* mRNA was measured in wild-type, *swi2-E834K*, and *swi2* disruption strains transformed with YCp plasmids containing either wild-type or mutant TBP. The TBP(V71E) mutant offered robust suppression of the *HO* expression defect in the *swi2-E834K* strain (Fig. 6D, compare lanes 7 and 9), while the TBP(N159K) mutant suppressed weakly (lane 10). Wild-type TBP on a low-copy-number plasmid did not suppress (lane 8), nor did wild-type TBP on a high-copy-number plasmid (lane 11). This result argues against the possibility that the mutation results in increased levels of TBP protein and that it is the increased amount of TBP that facilitates *HO* expression in the *swi2-E834K* mutant. Introducing a plasmid with the wild-type *SWI2* gene restored normal *HO* expression in the *swi2-E834K* strain (lane 12). Although the TBP(V71E) mutant strongly suppressed the defect in *HO* expression in the *swi2-E834K* mutant, only a minor effect was seen in the strain with the *swi2*

grown on 5-FOA medium to eliminate the YCp-*URA3*-TBP(wild type) plasmid. Cells with the indicated TBP plasmid as the sole source of TBP in the cell were then grown in selective medium, and the RNAs were isolated for S1 nuclease protection assays to measure *HO* and *CMD1* (internal control) RNA levels. (D) Strains DY150 (wild type) and DY9726 (*swi2-E834K*) were each transformed with two plasmids, one with a *TRP1* marker and one with a *URA3* marker, as follows: lanes 1 and 7, pRS314 vector and pRS316 vector; lanes 2 and 8, YCp-TBP(wild type) and pRS316 vector; lanes 3 and 9, YCp-TBP(V71E) and pRS316 vector; lanes 4 and 10, YCp-TBP(N159K) and pRS316 vector; lanes 5 and 11, YEp-TBP(wild type) and pRS316 vector; and lanes 6 and 12, pRS314 vector and YCp-*SWI2*. The cells were grown on selective medium to maintain both plasmids, and RNAs were isolated for S1 nuclease protection assays to measure *HO* and *CMD1* (internal control) RNA levels. (E) Strains DY150 (wild type), DY4394 (*ash1*), DY6806 (*spt3*), DY9726 (*swi2-E834K*), DY9711 (*swi2-E834K ash1*), and DY9709 (*swi2-E834K spt3*) were each transformed with either YCp-TRP1-TBP(wild type) or YCp-TRP1-TBP(V71E). The cells were grown on selective medium to maintain the plasmid, and RNAs were isolated for S1 nuclease protection assays to measure *HO* and *CMD1* (internal control) RNA levels. (F) Ribbon diagram of the TBP structure (49, 50), with the V71, N159, and G174 residues highlighted.

gene disruption (data not shown). We conclude that the V71E substitution in TBP allows *HO* expression, despite a partially defective Swi2 protein.

The TBP(V71E) and TBP(N159K) mutations act dominantly, as the chromosomal *SPT15* gene encoding TBP is still present. We attempted to address whether these mutations would have a stronger effect on *HO* expression if present as the sole source of TBP in the cell. However, plasmid shuffle experiments (data not shown) demonstrated that cells (*SWI2* or *swi2-E834K*) are not viable with a YCp plasmid with TBP(V71E) or TBP(N159K) as the sole source of TBP, consistent with earlier studies with TBP(V71E) (44, 71). However, a more modest substitution in TBP of valine to alanine at position 71 is viable as the sole copy of TBP (4). Interestingly, TBP(V71A) did not promote *HO* expression in the presence of the defective Swi2(E834K) (Fig. 6C, lane 3). Similarly, other substitutions at residue 159 of TBP, N159D or N159L, were viable as the sole copy of TBP but were ineffective in activating *HO* in the *swi2-E834K* strain (Fig. 6C, lanes 4 and 5).

We then asked if the TBP(V71E) mutation could augment the suppression of the *swi2-E834K* mutation by *spt3* or *ash1*. The wild type and *ash1* and *spt3* mutants, which were either wild-type *SWI2* or *swi2-E834K*, were transformed with plasmids carrying wild-type TBP or TBP(V71E). *HO m*RNA was then measured in these strains. The *ash1* and *spt3* mutations again provided partial suppression of *swi2-E834K* (Fig. 6E, lanes 4 to 6). More importantly, the TBP(V71E) mutation in conjunction with the *ash1* mutation provided stronger suppression than either mutation alone (Fig. 6E, compare lanes 5 and 11). In contrast, combining TBP(V71E) and *spt3* did not show a strong additive effect. This lack of additivity is consistent with the idea that Spt3 affects transcription by regulating TBP binding. However, the strong additive effect of combining the *ash1* mutation with TBP(V71E) suggests that these two mutations suppress the *HO* expression defect of the *swi2-E834K* mutant by distinct mechanisms.

DISCUSSION

The SWI/SNF chromatin-remodeling complex is required for expression of the yeast *HO* gene, and we investigated SWI/SNF binding to *HO* using chromatin immunoprecipitation assays. A mutation affecting the Gcn5 histone acetyltransferase eliminated both SWI/SNF binding and *HO* expression, whereas a mutation in the *ASH1* repressor rescued both of these defects. This suggests that SWI/SNF binding is stabilized by histone acetylation and that *GCN5* and *ASH1* act in opposition in regulating SWI/SNF binding. The fact that a mutation in the Sin3-Rpd3 histone deacetylase restored *HO* expression and SWI/SNF binding despite the absence of Gcn5 supports the idea that histone acetylation regulates SWI/SNF binding. Our genetic experiments have shown that a *swi2-E834K* hypomorphic allele can be suppressed either by a TBP point mutation or by deletion of *SPT3*, encoding a TBP-interacting factor. These results suggest that SWI/SNF promotes TBP binding at the *HO* promoter, although this may be indirect.

Interdependent binding of SWI/SNF and SAGA. ChIP assays demonstrated the ordered recruitment of transcription factors to the *HO* promoter (25). The Swi5 sequence-specific DNA-binding protein was shown to bind first, and then the SWI/SNF chromatin-remodeling complex, the SAGA complex with the Gcn5 histone acetyltransferase, and the SBF DNA-binding factor were recruited in sequence, leading to gene activation in mother cells (Fig. 7A). However, Ash1 was shown to bind to the URS1 region of the promoter in daughter cells and inhibit SWI/SNF recruitment and subsequent gene expression (25). Our data allow us to present a revised model for factor recruitment at the *HO* promoter (Fig. 7B). We showed that the Gcn5 HAT activity of SAGA is required for sustained binding by SWI/SNF. Ash1 and Sin3/Rpd3, either separately or as part of one complex, inhibit SWI/SNF binding to *HO* (Fig. 7B). Cosma et al. (25) showed that SWI/SNF is needed for SAGA binding (shown between SWI/SNF and Gcn5 in Fig. 7B). Thus, there is interdependence in the stable binding of SWI/SNF and SAGA to the *HO* promoter.

Swi5 interacts directly with SWI/SNF (66), and SAGA subunits were detected as interacting with Flag-tagged Swi5 (42). It is possible that Swi5 recruits both SWI/SNF and SAGA to *HO* (Fig. 7B), and thus, both chromatin remodeling and histone acetylation are required for these two complexes to associate stably at *HO*. Swi5 also interacts directly with Mediator, and both Swi5 and SWI/SNF are required for Mediator binding to the *HO* promoter (12). We speculate that there may also be interactions between SAGA and Mediator that stabilize the binding of both complexes (Fig. 7B), as there is interdependent binding to the *ARG1* promoter for SWI/SNF, SAGA, and Mediator (70).

In the sequence of events at the *HO* promoter, Swi5 is the first factor to bind, and it recruits the SWI/SNF and SAGA complexes that bind in a stable manner. These complexes remain bound to *HO* long after the unstable Swi5 factor is degraded. *HO* regulation is unusual in that *HO* expression occurs significantly later in the cell cycle than Swi5 binding, with the gene expressed in late G_1 phase when the Cdc28 cyclin-dependent kinase is activated. Swi5 appears to be absent from the nucleus at the time *HO* is transcribed (63). Thus, the *HO* promoter has been described as having a "memory" (25), which may consist of the stable, interdependent binding of SWI/SNF and SAGA.

Gcn5 is required for binding of the SBF factor at *HO* (25), and SBF is required for Mediator binding at the URS2 and TATA regions of the promoter (12, 24) (Fig. 7B). Importantly, the presence of Mediator at TATA is not sufficient for *HO* transcription, and activation of the Cdc28 cyclin-dependent kinase is required for recruitment of RNA Pol II (24). TBP binds very briefly to *HO* (85), and it appears that many factors are required for TBP binding (Fig. 7C). SWI/SNF, SAGA, and Mediator are all required for TBP binding at other promoters (69), and thus, they are shown as possibly stimulating TBP binding at *HO*.

Sequential recruitment of transcription factors. We find that the Gcn5 histone acetyltransferase activity is required for SWI/SNF to stably associate with the *HO* promoter, at least in the presence of the Ash1 negative regulator. Sequential recruitment of transcription factors has also been seen at other genes, including the human beta interferon, α1 antitrypsin, collagenase, and PPARγ2 genes (2, 58, 73, 77), and for each of these promoters, binding of a Gcn5-like histone acetyltransferase precedes SWI/SNF binding (2, 58, 73, 77). It has also been shown that histone acetylation precedes SWI/SNF bind-

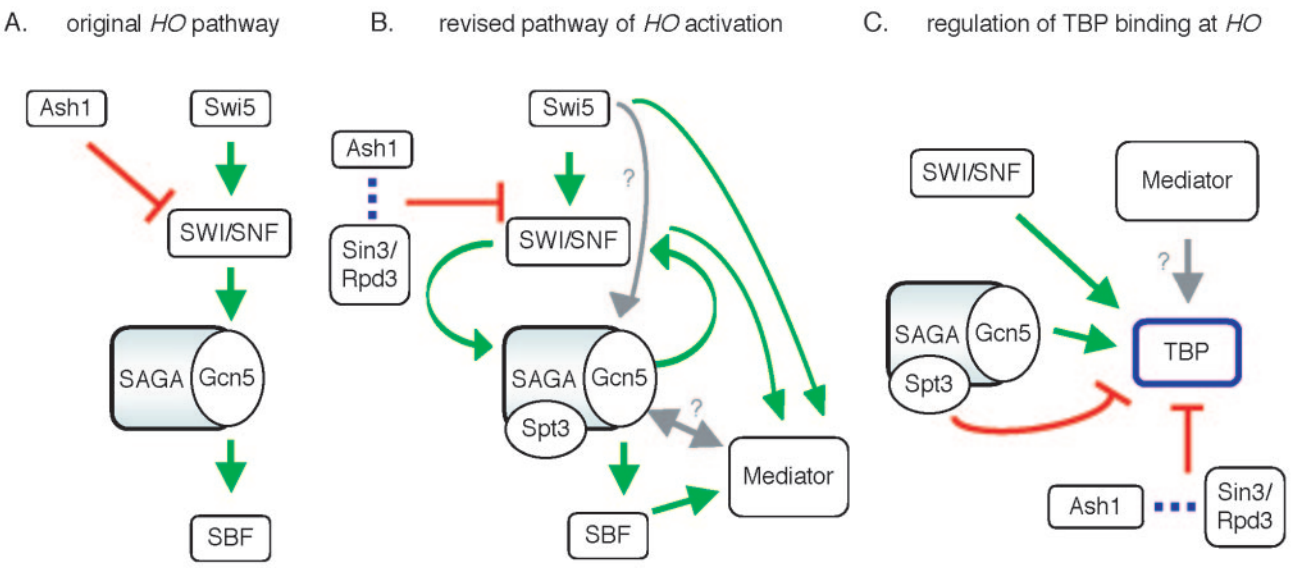


FIG. 7. Proposed model of *HO* regulation. Green arrows represent stimulation or recruitment, red bars represent inhibition, and gray arrows represent hypothetical interactions. (A) Original model for factor recruitment to *HO* based on Cosma et al. (25). In mother cells, Swi5 recruits SWI/SNF, which recruits SAGA (with Gcn5), and Gcn5 is required for SBF binding. Ash1 inhibits SWI/SNF binding in daughter cells. (B) Revised model for factor recruitment to *HO*. Swi5 recruits SWI/SNF and possibly SAGA to *HO*, and SWI/SNF and SAGA are mutually required for stable binding to the promoter. Swi5 and SWI/SNF are both required for Mediator binding to the URS1 region of the promoter. Ash1 and Sin3/Rpd3 inhibit SWI/SNF binding to *HO*; the dashed line between Ash1 and Sin3/Rpd3 indicates that, despite the fact that they can be found in the same complex, *ash1* and *sin3* mutations have additive effects on the regulation of *HO*. In the URS2 region, Gcn5 is required for SBF binding and SBF is required for Mediator binding. SAGA and Mediator have been shown to stimulate each other's binding at some promoters, and a hypothetical interaction is indicated. (C) Regulation of TBP binding at *HO*. SWI/SNF and Gcn5 both stimulate TBP binding to *HO*, and Spt3 and Ash1-Sin3/Rpd3 inhibit TBP binding. A molecular role for Mediator in stimulating *HO* transcription has not been clearly defined, and it may also stimulate TBP binding.

ing at the myogenin promoter (29) and that a *gcn5* mutation reduces the rate at which SWI/SNF is recruited to the *SUC2* promoter following induction (35). Govind et al. (37) recently showed that the Gcn4 activator recruits SWI/SNF, SAGA, and Mediator simultaneously to the *ARG1* promoter and that a *gcn5* mutation reduces SWI/SNF occupancy at *ARG1*. Thus, *HO* regulation conforms to the prevalent observation in that histone acetylation promotes stable binding of SWI/SNF.

Gcn5 overcomes Ash1 inhibition of SWI/SNF binding. Our results suggest that Ash1 acts as an inhibitor of SWI/SNF binding and that Gcn5 is required to overcome Ash1-mediated repression (Fig. 7B). At first glance, this role of Gcn5 is difficult to explain, as *HO* is expressed only in mother cells and Ash1 is a daughter-specific repressor (14, 76). However, Ash1 is not localized exclusively in daughter cells. Sil and Herskowitz (76) used immunofluorescence microscopy to quantitate Ash1 localization and found that 73% of large-budded cells had Ash1 visible only in daughter cells, while 17% of large-budded cells showed Ash1 visible in both mother and daughter cells. This suggests that Ash1 is present in both mother and daughter cells, with substantially more protein present in daughters. Ash1 is an unstable protein (60), and when sufficient Ash1 is degraded in mother cells, it will be present, but below the limit of detection.

The idea of Ash1 repression in mothers is supported by mating-type-switching experiments, a single-cell bioassay that assesses *HO* expression in mother versus daughter cells (14). Absolutely no mating-type switching is seen wild-type daughter cells, but an *ash1* mutation results in switching the mating type in daughter cells. Importantly, switching goes from 70% in *ASH1* mother cells to 100% in *ash1* mothers. This increased switching frequency in *ash1* mothers demonstrates that Ash1 does indeed inhibit *HO* expression in mother cells. Supporting this idea, an *ash1* mutation increased the fluorescence intensity of the *ho*::GFP-NLS-PEST reporter in mother cells (data not shown). Finally, the *ho*::GFP-NLS-PEST reporter was expressed in mother cells in the *gcn5 ash1* mutant (Fig. 3), despite the absence of the normally required histone acetyltransferase, demonstrating that Ash1 does inhibit *HO* expression in mother cells. Thus, we conclude that Ash1 inhibits SWI/SNF binding in mother cells and that Gcn5 is required to overcome this repression and allow *HO* expression.

Histone acetylation and regulation of *HO* transcription. We have shown that *HO* expression in the *swi5 sin3* mutant is dependent on SWI/SNF and that SWI/SNF binds to the *HO* promoter in the *swi5 sin3* strain (Fig. 1). Thus, the requirement for the Swi5 activator can be bypassed by a *sin3* mutation. Acetylation of nucleosomes facilitates stable in vitro binding of the SWI/SNF complex to nucleosomal templates (39), and it is plausible that the increased histone acetylation in the *sin3* mutant allows SWI/SNF binding in the absence of recruitment by Swi5. Additionally, *HO* is expressed in the *gcn5 sin3* double mutant, and SWI/SNF binds to the promoter at wild-type levels. As acetylation appears to be necessary for stable SWI/SNF association with *HO*, other HATs may be acetylating the *HO* promoter in the *gcn5 sin3* strain. These HATs could be re-

cruited to the promoter, possibly by Swi5, or their effect may be a consequence of untargeted global action (83), which is accentuated in the absence of the deacetylase activity.

It has recently been demonstrated that Ash1 is present in the large Sin3-Rpd3 histone deacetylase complex (19). Thus, the Ash1 DNA-binding protein may recruit the Sin3-Rpd3 HDAC to the *HO* promoter, providing a mechanism for transcriptional repression by Ash1. We have shown that suppression of the *HO* expression defect of the *gcn5* mutation by the *sin3* and *ash1* mutations is additive (Fig. 4A), implying that Sin3 and Ash1 have independent mechanisms of repression. Moreover, *sin3* and *ash1* mutations have distinct effects in suppressing the *HO* expression defect of the *swi2-E834K* mutation. *HO* was expressed in a *swi2-E834K ash1* strain (Fig. 6E) but not in a *swi2-E834K sin3* mutant (Fig. 6A). Thus, Ash1 and Sin3/Rpd3 have distinct functions in repressing *HO* expression, despite their presence in the same protein complex. Further work is needed to parse the roles of Ash1 and Sin3/Rpd3 in regulating *HO* transcription.

SWI/SNF regulates TBP binding at the *HO* promoter. DNA binding by TBP is thought to be the limiting event in transcriptional activation, as TBP binding correlates with transcriptional activity (54, 55). In vitro studies show that although TBP and TFIIA easily bind a TATA element in naked DNA, they cannot bind to a TATA site assembled within a nucleosome (43). However, TBP and TFIIA binding in vitro to a nucleosomal TATA can be seen if the SWI/SNF remodeler is included in the reaction (43) or if the histones in the nucleosomal template are hyperacetylated (13). We have detected SWI/SNF binding to the TATA region of the *HO* promoter using a ChIP assay (Fig. 5). Although less SWI/SNF binding is seen at TATA than at URS1 or URS2, it may be that SWI/SNF binds stably at URS1 and URS2 but only transiently at TATA. Indeed, TBP association with the *HO* TATA is very brief during the cell cycle (85). It is also possible that looping between the TATA region and the upstream promoter DNA brings SWI/SNF to the TATA region. Future work involving chromatin conformation is needed to address these questions. This SWI/SNF binding at TATA is consistent with a role for SWI/SNF in facilitating TBP binding to the *HO* promoter.

Our genetic studies support a role for SWI/SNF in stimulating TBP binding to the *HO* promoter (Fig. 7C). Spt3 is a subunit of the SAGA complex that contains Gcn5 (79), and Spt3 interacts genetically and physically with TBP (31). Spt3 has different roles at different promoters, acting either positively or negatively, depending on the specific promoter (8–10, 85). An *spt3* mutation reduces TBP binding to a number of promoters, including *GAL1* and *PHO5* (8, 10, 30). In contrast, an *spt3* mutation stimulates TBP binding to *HO* and allows *HO* expression in the absence of Gcn5 (85). Here, we find that an *spt3* mutation partially restores *HO* transcription despite the defective SWI/SNF complex in the *swi2-E834K* mutant. This suggests that SWI/SNF and Spt3 act antagonistically, with SWI/SNF stimulating and Spt3 inhibiting TBP binding to *HO* (Fig. 7C).

We also show that dominant gain-of-function substitutions in TBP, such as TBP(V71E) and to a lesser extent TBP(N159K), overcome the defect in *HO* transcription caused by the *swi2-E834K* mutation. Previous studies have shown that the TBP(V71E) mutant shows increased basal transcription, even though activated transcription was unaltered (44). An arginine substitution at V71 has also been shown to increase basal transcription and overcome Ssn6-Tup1- and Sin3-Rpd3-mediated repression (34, 44). The fact that amino acid substitutions in TBP allow *HO* expression in the *swi2-E834K* mutant suggests that the mutant TBP molecule may be impervious to negative regulation, thereby allowing *HO* transcription despite a defective SWI/SNF coactivator.

The V71 and N159 residues are both located on the concave, or DNA-binding, surface of TBP (Fig. 6F) (49, 50). Additionally, this region of the TBP molecule has also been implicated in negative regulation by inhibition of DNA binding by TBP by several different mechanisms (68). TBP forms homodimers that are inhibitory to DNA binding; mutations in the dimer interface (the region including V71 and N159) can result in increases in basal gene expression. While a V71R substitution markedly reduces dimerization, V71E does not (44). TAF1 inhibits DNA binding by TBP via the TANDI segment of TAF1, which interacts with the same surface of TBP (7, 52, 57). Importantly, the V71E substitution blocks TBP from binding to the N-terminal domain of Taf1 in vitro (21). We examined *HO* expression in a *swi2-E834K* mutant with a Taf1 mutant lacking this N-terminal domain, but no suppression was seen (data not shown). Mot1 is an ATPase and TBP-associated factor that uses ATP hydrolysis to disrupt TBP-DNA complexes in vitro, and thus, it can inhibit TBP binding to DNA (6, 67). Genetic and expression-profiling studies have suggested that Mot1 has both positive and negative roles in transcriptional regulation (3, 27, 33), and TBP(V71E) is defective for binding to Mot1 in vitro (26). We tested several *mot1* mutations, but they were unable to suppress the *HO* expression defect caused by *swi2-E834K* (data not shown).

The Spt3 subunit of SAGA interacts with TBP (31), and Spt3 inhibits TBP binding at *HO* (85). Importantly, an *spt3* mutation partially restores *HO* transcription in the *swi2-E834K* mutant (Fig. 6B). Spt3 is thought to interact with TBP via residue G174 (31) and thus not via the concave surface that contains V71 and N159. Indeed, a TBP(G174E) substitution believed to reduce interaction with Spt3 also allows *HO* expression in the *swi2-E834K* mutant. However, it is possible that Spt3 interacts with TBP via both G174 and the concave surface; in support of this, we do not see significant additive suppression from combining an *spt3* mutation with the TBP(V71E) substitution. Further work is needed to test this hypothesis. The suppression of the *swi2-E834K* mutation by *spt3* and by substitutions in TBP suggests that SWI/SNF stimulates TBP binding at *HO* while Spt3 inhibits it. Therefore, we propose a model in which Spt3 and another repressor molecule, possibly Ash1 with Sin3/Rpd3, inhibit TBP binding to the *HO* promoter and the SWI/SNF coactivator overcomes this repression, facilitating TBP binding. Finally, TBP(G174E) also allows *HO* expression in strains with a *gcn5* null mutation (85). This common suppression by TBP(G174E) suggests that SWI/SNF and SAGA function in a common pathway, and we suggest that SWI/SNF and SAGA both stimulate TBP binding (Fig. 7C). There is strong evidence supporting a role for the Gcn5 histone acetyltransferase in SAGA stimulation of DNA binding by TBP and TFIIA (13), and we suggest that SAGA stimulates TBP binding at the *HO* promoter. Importantly, an *spt3* mutation both increases TBP binding at *HO* and allows

HO expression in the absence of the Gcn5 HAT (85). Finally, it is not known how Mediator promotes *HO* transcription, and it is possible that Mediator facilitates TBP recruitment.

Regulation of TBP binding by SWI/SNF and SAGA may be a quite general phenomenon. Targeted recruitment of Rpd3 to promoters did not reduce the binding of DNA-binding activators but did inhibit the binding of SWI/SNF and SAGA (28). Thus, the decreased histone acetylation affected SWI/SNF and SAGA occupancy, which is not surprising, as both of these complexes contain bromodomains that recognize acetylated lysines. Importantly, targeted Rpd3 recruitment also resulted in decreased binding of TBP at the promoter. Because activator binding was unaffected, one can conclude that the activator was not sufficient to recruit TBP. Thus, DNA binding by TBP in vivo requires either histone acetylation, the presence of SWI/SNF and SAGA, or both.

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